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13. ABSTRACT (Maximum 200 words) The purpose of this project is to examine racial differences in body composition among black, white, asian and other ethnic groups in U.S. military women. Expected outcome is the development of ethnic specific prediction equations for body composition assessment; specifically percent body fat (% fat). To date, data collection includes 128 white women and 111 black women who participated in a multi-compartment assessment of fat free mass (FFM) and % fat. In addition to the multi-compartment assessment each woman was measured using standard anthropometric techniques for body circumferences, skeletal breadths and skinfold thickness. Preliminary statistical analysis indicates no significant differences between Blacks and Whites on the contribution of various anthropometric measurements to % fat. Further data collection on other ethnic groups is needed, however, the early results suggest that prediction equations derived from these anthropometric variables should not contain systematic differences due to ethnicity.				
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FOREWORD

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IV. INTRODUCTION

All branches of the military have established standards for accession and retention (1-3). The accession standards are based on indirect determinations of body composition from weight for height (W/H) tables, while the retention standards include an assessment of a body composition based on W/H measurements and a test of aerobic fitness. Periodic review of W/H is conducted within all branches of the armed forces. Failure to meet these standards results in anthropometric assessment and determination of percent body fat (% BF) from regression equations based on circumference measurements. However, Vogel et al (4) reported that due to difficulties encountered in predicting body density in African-American females, primarily hydrophobia, the equation selected for use with females was developed from the White population studied. *This means that for technical reasons, the population used to develop the current Army equation did not contain any minority women. This also raises the question of the appropriateness of this equation for broad use within an Army where 53% of the females soldiers are members of minority ethnic groups.*

If the soldier has a higher %BF than allowed, then she undergoes medical review, is assigned to a supervised program of diet and exercise, and is given a set period of time prior to final evaluation. If the soldier does not meet the retention standards, then she is separated from military service. Such a separation results in a loss for both the Armed Forces in terms of training and knowledge lost to the military and for the individual in terms of the loss of a career and benefits. Therefore, it is vital to be certain that the standards selected are fair and unbiased. *If there are age, or ethnic-related biases in the regression equations that estimate %BF, it would be essential that these biases be scientifically quantified, in order to account for them in all retention evaluation procedures.*

The experiment outlined below proposes: to determine the accuracy and precision of the Army and Navy equations to predict percent body fat in minority and non-minority female soldiers across representative ranges of age and body fat; to develop new prediction models using a modern, nonparametric tree-structured model that will be applicable to minority and non-minority female soldiers across all ages and ranges of body fat; and to test the validity of the new prediction models using cross-validation, a computationally-intensive technique.

The recommendation to conduct research into the area of health promotion and disease prevention among military women, is included among those published by the Institute of Medicine in 1995 (6). If the current W/H, body fat and fitness standards of the military were met, then female soldiers would be free of diseases related to overweight and obesity. *In order to ensure a healthy female component of the military it is vital that all female soldiers meet the fitness and body composition standards and that these standards be uniformly applicable across all ethnic groups, ages and ranges of body fatness.*

The results of the proposed experiment will provide the Armed Forces with a scientifically based litmus test of the equations currently being used to estimate %BF, to determine promotion rate and/or retention in the armed forces, and to ensure the health promotion and disease prevention of all minority and non-minority females soldiers.

V. BODY OF PROPOSAL

A. BACKGROUND

Retention standards for all branches of the military include an assessment of a body composition based on weight for height measurements (W/H) and a test of aerobic fitness (1-3). Periodic review of W/H is conducted and failure to meet these standards results in an anthropometric assessment of %BF. If the soldier has a higher %BF than allowed, then she undergoes medical review, is assigned to a program of diet and exercise, and is given a set period of time prior to final evaluation. Separation from the armed service results, if the retention standards are not met.

The current equations to predict %BF, based on circumference measurements, were generated on populations of the services that reflected the proportion of ethnicity of the services in the late 1980's. For example, the Army validation experiment was performed in a population partitioned in the following manner: 21% of the population studied were female and of the females 38% were African-American (AA). In 1995 (5) 53 % of all female soldiers are minority group members and 44% of all female soldiers are AA. These figures illustrate the growing contribution of minority women to the Army.

At that time the only criterion method used was hydrostatic weighing which, because of differences between ethnic groups in bone density, is known to have limited use in a minority populations. Vogel et al (4) reported that due to the higher prevalence of hydrophobia among AA soldiers than among the white soldiers it was difficult to predict body density in AA female soldiers. Therefore, the equation selected for use with females was developed from the population of white female soldiers studied. *This means that for technical reasons, the population used to develop the current Army equation did not contain any minority women.*

Since then, dual energy x-ray absorptiometry (DXA) has become widely available, which overcomes some of the theoretical and practical problems of underwater weighing, in that it quantitates bone density and bone mineral content. In combination with the measurement of body volume from hydrostatic weighing and total body water measurement by isotope dilution, DXA can be used in a four compartment model of body composition validated by Heymsfield et al. (6). *This four compartment model has replaced hydrostatic weighing as the criterion method for determining body composition and accommodates differences between minority groups in bone density and appendicular muscle density.*

Since 44% of the female soldiers in the Army are African-American, the literature on differences between AA and whites is briefly reviewed. Differences in components of body composition between African-Americans and whites have been known for almost four decades (7). The best documented difference in body composition between these two ethnic groups is an increased density of the fat free mass (FFM) in African-Americans because of a heavier and denser skeletal mass, and denser appendicular muscle mass (8-11). In 1990, Zillikens and Conway (12) suggested a difference in location of adipose tissue stores, specifically that African-American women have greater upper body obesity as compared to white women based on skinfold thickness ratios. A larger subscapula skinfold has been reported in a large population of African-American women, who are described in the CARDIA study (13). Apparently this centrality of fat deposition patterns begins in childhood, since AA have a more central pattern of fat deposition than white children (14). The fat distribution differences between AA and white children are evident in the preschool period and through adolescence into adulthood. Mueller (14) further reported that the ethnic trends of fat

deposition patterns were independent of fatness level, socioeconomic status, and age, suggesting a possible genetic component to fat deposition patterns. *Because of these differences in fat deposition patterns, an ethnically based regression equation or a regression model that can account for ethnic differences is needed.*

A major objective of the present study is to evaluate the existing Army and Navy models for predicting body fat and to develop new models for predicting body fat and lean body mass that adequately accommodate minority and non-minority military women across all ranges of age and body fat.

B. HYPOTHESES

1. The Army and Navy regression equations for estimation of percent body fat apply to minority and non-minority military or military-eligible females across all applicable ranges of age and body fat with less than 5 soldiers out of 100 misclassified for retention.
2. The agreement between the Army and Navy regression equations and the four compartment model criterion method will show an acceptable concordance correlation.
3. The new prediction equations for estimation of percent body fat apply to minority and non-minority military or military-eligible females across all applicable ranges of age and body fat with less than 5 soldiers out of 100 misclassified for retention.
4. The accuracy and precision of the new equations for predicting the body fat or lean body mass developed from the four compartment criterion method will be acceptable based on the concordance correlation coefficient.

C. TECHNICAL OBJECTIVES

1. To determine the accuracy and precision of the Army and Navy equations to predict percent body fat in minority and non-minority female soldiers across all ages and ranges of body fat.
2. To develop new prediction models using a modern, nonparametric tree-structured model that will be applicable to minority and non-minority female soldiers across all ages and ranges of body fat.
3. To test the validity of the new prediction models using cross-validation, a modern computationally-intensive technique.

D. SUBJECT SELECTION

This study will include a total of 250 normal weight women, selected according to the following

admission criteria:

- Age range 17-40
- Body weight based on Army retention criteria
- Broadly representative of the ethnic composition of the Armed Forces, which in 1995 was: 40% minority (31% African-American, 5% Hispanic, 2.5% Asian-American/Pacific Islander, and 1.5% Native American) and 60% non-minority.
- Broadly representative of the age distribution of the Armed Forces, which in 1995 was: ≤ 20 y, 15.6%; 21-25 y, 31.6%; 26-30y, 20.3%; 36-40y, 10.8%; 41-45 y, 4.4%; >45 y, 1.3%.
- Members of the military and/or military-eligible
- Absence of hydrophobia
- Free of major metabolic disease such as: diabetes and cardiovascular disease

Recruitment and medical screening will be conducted by a research assistant under the close supervision of the principal investigator. Participants will be recruited from members of the armed services in the metropolitan San Diego, CA area, by means of ads placed in local editions of the armed forces newspapers and in the San Diego newspaper.

E. SAMPLE SIZE

Since the goal of this experiment is to test and develop prediction equations and not to test a true null hypothesis, standard power analysis does not apply. Therefore sample size was determined based on the standard requirement of having at least 10 subjects per predictor variable to develop a multiple regression equation. We have approximately 25 predictor variables (see planned analysis section). Consequently, we have chosen a sample size of 250 military or military-eligible women with an approximate population distribution 149 whites and 101 from other ethnic groups. In addition, Lohman (15) demonstrated that it is necessary to have at least 50 subjects per experiment to develop validation equations. The proposed sample size of 250 will include ~75 AA women and a total of 100 ethnic minorities, which would be sufficient to develop a separate ethnically based equation or model if needed.

F. BODY COMPOSITION MEASUREMENTS

1. Anthropometry

The anthropometric measurements will include skinfolds at the triceps, biceps, subscapula, and suprailiac and circumferences at the following sites: neck, upper arm, forearm, wrist, chest, abdomen (midway between the lowest rib and the iliac crest, 16), hip, thigh, and calf. These measurements will be made according to Lohman et al., (17). All available military service equations (4, 18, 19) will be tested.

2. Fat Patterning

The ratio of the triceps to subscapula skinfold thickness has been demonstrated to vary in ethnic groups (12), and Mueller (14) has reported that the subscapula skinfold thickness may be indicative of genetic differences in fat patterning. Other indices of fat patterning will also be determined, i.e., waist to hip ratio (WHR) which is the ratio between the abdominal circumference divided by hip circumference, and sagittal diameter (SagD) using the Holtain abdominal caliper at the site of the fourth lumbar (20). Both of these have been reported to be anthropometric surrogates for visceral adipose tissue in minority and non-minority women (20-23). All of these anthropometric indices of fat patterning will be included as a predictor variable offered to the CART procedure.

3. Isotope Dilution

Each subject will report to the Human Study Facility by 7:00 A.M. after a twelve hour overnight fast. Weight will be determined to ± 0.001 kg, height will be determined to ± 0.01 cm. by means of an Holtain stadiometer. A 20g dose of 99.975% deuterium oxide ($^2\text{H}_2\text{O}$) administered upon arrival in the lab and after the collection of a respiratory water sample. Additional samples will be collected at approximately 3 and 4 hours afterwards. The samples will be analyzed using a Miran (The Foxboro Co., Norwalk, Conn.) infrared spectrophotometer. Percent body fat from D_2O will be calculated using standard isotope dilution equations. Total body water will be determined in liters. Correction will be made for non-aqueous exchange of hydrogen, isotopic fractionation. All values will be converted to kilograms using the constant 0.009934. This method has a day to day CV of $<2.5\%$.

4. Hydrodensitometry

Body density will be measured by using a hydrodensitometry system described previously (24). After complete submersion and maximal expiration by the subject underwater weight will be recorded by four platform force transducers. The subjects will be asked to perform three training trials and then three additional trials. The underwater weight will be recorded as a average of the last three trials. Density will be corrected for residual lung volume which will be measured simultaneously with underwater weight by means of a closed system nitrogen dilution system.

5. Dual Energy X-ray Absorptiometry

The dual energy x-ray absorptiometer (DXA) consists of a scan table, a computer monitor, a keyboard, and a printer. The scan table contains the x-ray generator, detector, and system electronics used to process the signals from the detected x-rays. One scan gives measurements of % BF, fat and lean tissue mass, and bone density and bone mineral content. Each scan takes approximately 20-30 minutes depending on the body composition of the individual. The radiation dose of 0.024-0.06 mRem is 1/800-1/1000 of the radiation received from a single chest x-ray exposure (40 mRem) or to whole body dose incurred each year due to cosmic radiation and natural radiation sources such as radon, thorium and potassium.

Measurements will be done using the Lunar DXA scanner. The subjects will be asked to change into a hospital gown and remove any metal objects to avoid possible attenuation of x-ray beam from watches, zippers, buckles and buttons. Any subjects with surgically placed metal implants or shrapnel will be excluded. In addition, the machine can only scan individuals between 25-196 cm long and 36-113 kg in weight. The subject lies in the supine position on the scanning table. Measurements will be taken at the speed appropriate for their body composition and will take 15 to 30 minutes and the subject receives up to 0.06 mrems of radiation. All measurements will be done by the same technician to avoid variability.

G. Four-Compartment Model

This model is an extension of one of Siri's models (25):

Body Weight = Aqueous weight + Mineral weight + Protein weight + Fat weight
and

Fat Free Mass = Aqueous weight + Protein weight + Mineral weight

where aqueous weight can be determined by isotope dilution, mineral weight from DXA, body density and body volume from hydrodensitometry; fat mass or fat free mass can then be obtained

using the algebraic equations given by Heymsfield et al., (6).

H. PRELIMINARY DATA

To date data have been collected on 239 white and black individuals. An analysis was undertaken to determine whether or not ethnic differences could be detected in the relationships between anthropometric variables and percent fat derived from a 4-compartment analysis. Anthropometric measures (14 girths, 9 skinfold thicknesses, and 5 diameters) were collected from 239 women, 128 white and 111 black. Percent body fat was determined from a 4-compartment analysis consisting of total body water measurement using whole body electrical impedance measured at 50 kHz and the predictive equations of Kushner and Schoeller; body bone mineral content from DXA (Hologic 1500W), and body volume from hydrodensitometry. Physical characteristics are provided in Table 1.

Table 1. Participant Characteristics

	Whites (n = 128)	Blacks (n = 111)	Total Sample
Age (yr.)	31.2 (7.0)	29.4 (6.8)*	30.3 (7.0)
Stature (cm.)	164.3 (6.4)	164.8 (5.9)	164.5 (6.1)
Mass (kg.)	67.5 (10.7)	70.4 (10.4)*	68.9 (10.7)
Body Fat (% of mass)	29.0 (6.4)	30.3 (6.9)	29.6 (6.6)

*differs significantly from whites (p < 0.05)

Effects of ethnic group membership on relationships between anthropometric variables and body fat content were investigated using the general linear model (MANOVA procedure in SPSS). Slopes and intercept values of the regressions of anthropometric variables on percent body fat from 4-compartment analysis were tested for differences associated with ethnic group membership. Results of the analyses for girth measurements are provided in Table 2. The table provides indications of the level of significance for the prediction of % body fat from the anthropometric measure, the R^2 value for the total model, that is the percent of variance accounted for by the anthropometric predictor, ethnicity (intercept affects), and the interaction between ethnicity and the anthropometric predictor (slope affects), and significance indicator for ethnic affects on the slope and intercept of the regression line.

Table 2. Affects of Ethnic Group on Prediction of % Fat from Girth Measurements

Girth Measure	Regression	R^2 for total model	Ethnic Group Affects	
			Slope	Intercept
Head	**	0.04	NS	NS
Neck	**	0.27	NS	NS
Shoulder	**	0.38	NS	NS
Flexed Arm	**	0.46	NS	NS
Relaxed Arm	**	0.52	NS	NS
Forearm	**	0.25	NS	NS
Wrist	**	0.12	NS	NS
Chest 1	**	0.49	NS	NS
Abdomen 1	**	0.69	NS	NS
Abdomen 2	**	0.64	NS	NS
Hips	**	0.63	NS	NS
Subgluteal Thigh	**	0.62	NS	NS
Mid-shaft Thigh	**	0.48		NS

Calf	**	0.25	NS	NS
Ankle	**	0.10	NS	NS

* significant with $p < 0.05$

** significant with $p < 0.01$

For each of the girths measured, there were no ethnic effects on the regression of the girth measure on percent body fat. It can be seen that there was a great deal of variation in the strengths of the associations between the measured girths and percent body fat. The strongest associations were for the abdominal girths, hips, and sub-gluteal thigh.

Table 3 presents the results of the analyses of the regressions between skinfold thickness measures and percent body fat. The table is constructed in the same way as Table 2.

Table 3. Affects of Ethnic Group on Prediction of % Fat from Skinfold Measurements

Skinfold Site	Regression	R ² for total model	Ethnic Group Affects	
			Slope	Intercept
Biceps	**	0.43	0.06	NS
Triceps	**	0.64	NS	NS
Chest	**	0.31	NS	NS
Subscapular	**	0.57	NS	NS
Midaxillary	**	0.52	NS	NS
Abdominal	**	0.52	NS	NS
Iliac Crest	**	0.54	NS	NS
Supraspinale	**	0.50	NS	NS
Thigh	**	0.50	NS	NS
Medial Calf	**	0.47	NS	NS

* significant with $p < 0.05$

** significant with $p < 0.01$

As with the girth measurements, there are no significant affects of ethnic group on the prediction of percent body fat. However, it should be noted that ethnic differences in the slope of the regression line approached significance for the biceps skinfold relationship. All the skinfold thickness models appeared to account for a similar percentage of the sample variance.

Table 4 contains the results of the analyses of the regressions of body diameters on percent body fat. This table is laid out in the same fashion as tables 2 and 3.

Table 4. Affects of Ethnic Group on Prediction of % Fat from Body Diameter Measurements

Diameter	Regression	R ² for total model	Ethnic Group Affects	
			Slope	Intercept
Biacromial	**	0.05	NS	NS
Chest A/P	**	0.10	NS	NS
Biliac	**	0.34	NS	NS
Wrist	NS	0.02	NS	NS
Hand	NS	0.01	NS	NS
Bitrochanteric	**	0.38	NS	NS
Ankle	NS	0.02	NS	NS

* significant with $p < 0.05$

** significant with $p < 0.01$

We can see from Table 4 that with the exception of biiliac and bitrochanteric measures, diameters are poor predictors of percent body fat. This is not surprising, since they are usually used as measures of skeletal size. Again, there were no affects on the regressions associated with ethnicity.

These analyses suggest that, in general, equations derived from these anthropometric measures should not contain systematic differences in prediction associated with ethnicity. However, it appears that use of biceps skinfold thickness should be avoided until the near-significant difference in regression slope associated with ethnicity can be studied further. This should not present a problem, given the similar strengths of relationships between all the skinfold thickness measures and percent body fat.

I. STATISTICAL ANALYSIS

1. Data Analysis

The data will be entered and independently verified by assistants. Data will be analyzed using the multiple regression analysis and *where appropriate* the CART module of the SYSTAT package. These analyses will be done in conjunction and consultation with the statisticians at WHNRC and NHRC.

We will fit two regression trees and three classification trees. The two continuous response variables for the regression trees will be:

1. Percentage body fat from a four compartment model (6) and
2. Lean body mass.

The three categorical response variables for the classification trees are:

1. Binary variable for the *DoD-wide goal* for female soldiers of $\leq 26\%$ body fat (Army Regulation 600-9). The two levels are ≤ 26 and $>26\%$ body fat.
2. Five-level categorical variable for the *retention cutoffs* for each age groups: for 17-20 yr $\leq 28\%$ BF, for 21-27 yr between 28 and 30% BF, for 28-39 yr between 28 and 30% BF, and for 40 & older $> 34\%$ BF.
3. Six-level categorical variable including *the DoD goal* and the *retention cutoffs*: $\leq 26\%$ body fat, for 17-20 yr between 26 and 28% BF, for 21-27 yr between 28 and 30% BF, for 28-39 yr between 28 and 30% BF, and for 40 & older $> 34\%$ BF.

All the regression and classification trees will use approximately 25 predictor variables:

- skinfold thicknesses measurements such as: triceps, biceps, subscapula, and suprailiac
- circumference measurements such as: neck, chest, upper arm, forearm, wrist, abdominal, hip, thigh, calf, including standard functions of them such as waist to hip ratio (WHR)
- Other anthropometric variables such as height and weight, including standard functions of them such as weight/height² or body mass index (BMI)
- Sagittal Diameter as measured by Holtain abdominal caliper
- Age
- Ethnic group
- Menstrual status
- Parity
- General health status

Statistical methods will also be used to evaluate the agreement between the actual percentage fat and that predicted by the Army and Navy regression models:

- For the comparison of two methods, Bland and Altman (26-27) recommend plotting the difference versus the mean. These *Bland-Altman plots* describe agreement better than conventional methods such as the Pearson correlation coefficient and linear regression.
- Lin (28) introduced the *concordance correlation coefficient* to quantify the agreement between two methods. The concordance correlation is the product of the Pearson correlation (measure of precision) and a bias correction factor (measure of accuracy). Lin (29) discusses statistical methods for evaluating the acceptability of the concordance correlation coefficient.
- The *misclassification error rate* corresponding to the various standard cutoffs for ascension and retention will be used to evaluate the practical effect of using the existing prediction equations. Misclassification error can be adjusted to allow for unequal costs of erroneously rejecting subjects and incorrectly accepting subjects.

Regression models will be developed to predict percentage fat and fat free mass from the anthropometric measurements. In addition, classification models will be developed to predict whether individuals fall in classes defined by the standard cutoffs for percentage fat. The conventional statistical methods for constructing these prediction models are multiple linear regression and discriminant analysis.

VI. CONCLUSIONS:

In general, the preliminary data suggest that %fat prediction equations developed from anthropometric measurements should not contain systematic differences due to ethnicity. The use of biceps skinfold thickness, at this time, should not be used until the trend toward a significant difference due to ethnicity can be further explored with the complete data set. Work that remains on this project is to focus on other ethnic groups, as outline in the original proposal, including Hispanic, Asian/Pacific Islanders, and Native Americans. This can be accomplished during this next year at no additional cost.

Following data collection on the other ethnic groups full statistical evaluation will be done. Based on the statistical consultation, at both the USDA, Western Human Nutrition Research Center and the Naval Health Research Center, it is possible that the analysis originally proposed by Dr. Conway (CART) is not entirely necessary. More indepth exploration of this issue will be pursued this next year.

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